(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(43) International Publication Date 21 July 2005 (21.07.2005)

PCT

(10) International Publication Number WO 2005/066341 A1

- (51) International Patent Classification⁷: C12N 11/00, 9/02, 9/34
- (21) International Application Number:

PCT/IN2004/000002

- (22) International Filing Date: 1 January 2004 (01.01.2004)
- (25) Filing Language:

English

(26) Publication Language:

English

- (71) Applicant (for all designated States except US): COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH [IN/IN]; (An Indian registered body incorporated under Regi, stration of Societies Act (Act XXI of 1860)), Rafi Marg, New Delhi 110 001 (IN).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): ABRAHAM, Tholath, Emilia [IN/IN]; Regional Research Laboratory (CSIR), Industrial Estate (PO), Trivandrum 695 019, Kerala (IN). BINDHU, Lekshmibai, Vasanthakumari, Amma [IN/IN]; Regional Research Laboratory (CSIR), Industrial Estate (PO), Trivandrum 695 019, Kerala (IN).
- (74) Agent: BHATNAGAR, Mahendra, Prasad; Lall Lahiri & Salhotra, Plot No. B-28, Sector 32, Institutional Area, Gurgaon 122 001, Haryana (IN).

- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with amended claims and statement

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR THE PREPARATION OF CROSS LINKED PROTEIN CRYSTALS

(57) Abstract: A protein such as an enzyme is immobilized by crosslinking crystals of the protein with a multifunctional crosslinking agent. The crosslinked protein crystals may be lyophilized for storage. A preferred protein is an enzyme such as amyloglucosidase, Horse radish peroxidase, plant peroxidases etc. Crosslinked enzyme crystals preferably retain at least 90% activity after incubation for three hours in the presence of a concentration of protease that causes the soluble uncrosslinked form of the enzyme to lose at least 92% of its initial activity under the same conditions. Enzyme crystals that are crosslinked may be microcrystals having a cross-section of 100microns or less. Crosslinked enzyme crystals are sturdy and can withstand harsh conditions and may be used for performing selective chemical reactions in organic or aqueous medium, in an assay, diagnostic kit or biosensor for detecting an analyte, in producing a product such as using crosslinked Peroxidase crystals to produce novel polymers, biotransformations including those used in industrial scale chemical processes and in environmental remediations.

